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This research investigated mitochondria as a potential energy source for compact power supplies, both as a battery and as a fuel cell. For the mitochondrial battery, a monolayer of mitochondria was constructed and the viability of mitochondria was tested by fluorescence microscopy. One battery employed the monolayer of mitochondria as a cathode and another battery employed the monolayer of mitochondria as an anode. Each battery was tested at low current discharge and the voltage was measured. The experimental results showed that the mitochondria can be used as an anode.
Mitochondria were also studied as a component of a fuel cell. Mitochondria were employed in anodic chambers of fuel cells. THe effects of an uncoupler and a mediator were tested. A mitochondrial fuel cell without a mediator delivered ~0.03 mA, whereas a cell with a mediator provided a maximum of ~0.065 mA current. The experimental results suggest that the cell performance depends on both diffusion of reduced mediator and electron transport between mitochondria and mediator.

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# QUANTITATIVE PREDICTION OF AVAILABLE POWER IN MITOCHONDRIAL ARRAYS FOR COMPACT POWER SUPPLIES


**Abstract**

This research investigated mitochondria as a potential energy source for compact power supplies, both as a battery and as a fuel cell. For the mitochondrial battery, a monolayer of mitochondria was constructed and the viability of mitochondria was tested by fluorescence microscopy. One battery employed the monolayer of mitochondria as a cathode and another battery employed the monolayer of mitochondria as an anode. Each battery was tested at low current discharge and the voltage was measured. The experimental results showed that mitochondria can be used as an anode. Mitochondria were also studied as a component of a fuel cell. Mitochondria were employed in anodic chambers of fuel cells. The effects of an uncoupler and a mediator were tested. A mitochondrial fuel cell without a mediator delivered ~0.03 mA, whereas a cell with a mediator provided a maximum of ~0.065 mA current. The experimental results suggest that the cell performance depends on both diffusion of reduced mediator and electron transport between mitochondria and mediator.


**Technical accomplishments**

1. Mitochondria as battery elements

1.1. Monolayer of mitochondria

The substrate for each mitochondrial monolayer was a gold-coated glass slide (GC slide). The GC slides were prepared using physical vapor deposition of gold on glass slides with nickel as an intermediate bond. The GC slides were incubated with CellTak (cell adhesive) solution to obtain CellTak coated gold-coated slides (CTGC slides). The viability of mitochondria as a monolayer was studied using JC1 assay. For this purpose, the CTGC slides were incubated with mitochondria solution containing the JC1 dye for 1 hour at 4°C. Mitochondria with intact membrane potential took up the JC1 dye and the dye accumulated in the mitochondrial matrix. The CTGC slides were rinsed with the phosphate buffer and placed in the mitochondria storage buffer. Each monolayer was imaged using fluorescence microscopy. This result confirmed that it is possible to obtain a viable monolayer of mitochondria.

1.2. Mitochondria as a cathode

The underlying hypothesis was that mitochondria might be used as a cathode by harnessing the excess protons in the inter-membrane space. When a mitochondrial array is coupled with a metallic anode such as zinc (standard reduction potential = -0.76 V), the electrons obtained from the anode could react with protons from the mitochondrial array. Such a system would be a metal-proton battery. This hypothesis was tested as follows. The monolayer of mitochondria was prepared as described above and imaged for testing the viability of mitochondria. The freshly prepared mitochondrial cathode was tested against a zinc anode. The electrolyte was mitochondria storage buffer containing mitochondrial nutrients such as pyruvate, a-ketogluterate, and NADH. The battery was subjected to low current discharge (100 nA) and the voltage was measured. A second battery without mitochondrial coating was also discharged as a control. The voltage profile of the cell with the mitochondrial cathode was below the voltage profile of the control cell. This result suggests that the monolayer of mitochondria may be used as an anode.

1.3. Mitochondria as an anode

Mitochondria may be used as an anode by harnessing the electrons from mitochondrial electron transport chain. These electron transport chains consist of enzyme complexes responsible for transporting electrons from one enzyme complex to the other. Electrons may be acquired from this process by applying an artificial electron acceptor such as PMS (Phenazine Methosulfate). The mitochondrial electrode (anode) was prepared as described above. The cathode in this case was a mixture of PMS and another irreversible reducing entity MTT. The electrolyte around the mitochondrial electrode was same as above, but for the addition of PMS. The open circuit potential measurement showed that the voltage from the mitochondrial anode was higher than the voltage from the control cell (CTGC without mitochondria).

2. Mitochondrial fuel cell

Mitochondria were employed in the anodic solution along with the nutrient succinate, the uncoupler 2,4 Dinitrophenol (DNP) and the mediator Phenazine Methosulfate (PMS). DNP prevented mitochondrial dysfunction due to respiratory control. The basic anodic buffer consisted of Potassium Phosphate ($K2HPO4$) and EGTA (Ethylene Glycol Tetraacetic Acid). The cathodic buffer was a plain Potassium Phosphate solution with Potassium Ferricyanide ($K3F(CN)6$). Anodic and cathodic compartments were separated using a cation exchange membrane.

In the anodic chamber, mitochondria consumed phosphate, succinate and DNP and transfered electrons to PMS. In the cathodic chamber, ferricyanide converted to ferrocyanide by accepting an electron. The electrons required for this reaction were supplied by the reduction of PMS in the anodic chamber. This reaction took place spontaneously when cations flew from the anodic chamber to the cathodic chamber. These cations were liberated in the anodic chamber by mitochondrial reaction. Theoretically, this system was able to deliver 1.7 mA current for one hour, whereas control cells (without mitochondria) were not capable of delivering any current.

For investigating the mitochondrial fuel cell performance, open circuit voltage and current discharge through a 1 KW resistor were measured. Mitochondrial fuel cells without PMS showed an open circuit voltage (OCV) of 0.4 – 0.45 V and delivered significant current (~0.03 mA) compared to control cells. The underlying mechanism of the cell operation is possibly the extraction of electrons from the electron transport chain in mitochondria through the permeable outer membrane. For the mitochondrial fuel cell with PMS, initial OCV was below that of the corresponding control cell. When compared with mitochondrial fuel cells without PMS, cells with PMS delivered substantial current (maximum of ~0.065 mA). This low initial OCV, and later gradual recovery, may be caused by oxidized species surrounding the electrode, suggesting a combined mechanism consisting of electron transport to PMS and diffusion of reduced PMS towards the electrode.